

Figure 1 Inhibition of labelled TSH binding to TSHR coated tubes by hMAb TSHR1 IgG and Fab. The control IgG was a human monoclonal autoantibody to GAD₆₅.

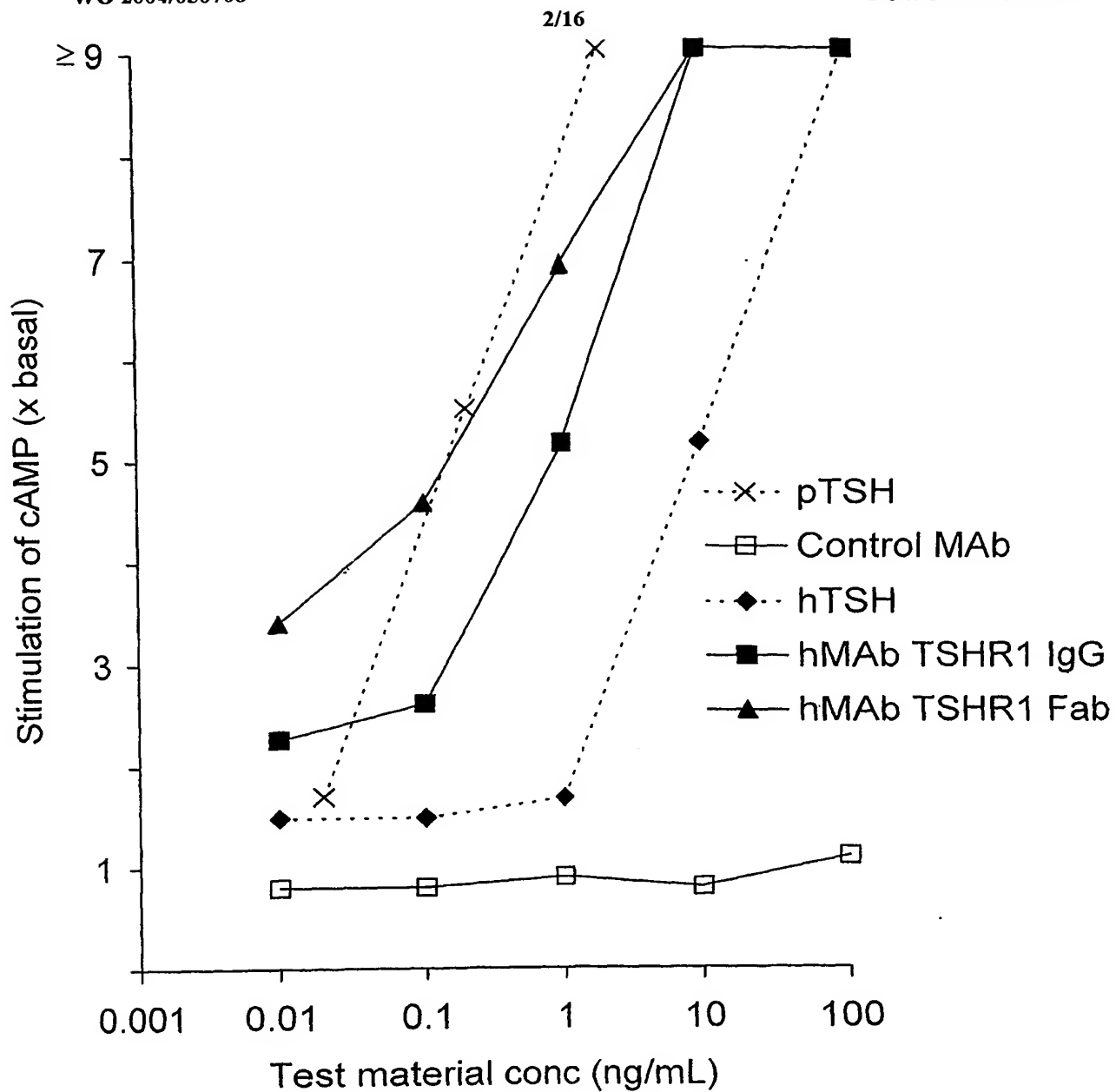


Figure 2 Thyroid stimulating activities of hMAb TSHR1 IgG and Fab, porcine TSH (70 units/mg; pTSH), recombinant human TSH (6.7 units/mg; hTSH) and a control monoclonal antibody (MAb: a human monoclonal autoantibody to thyroid peroxidase (2G4)). Basal = cAMP produced in the presence of NaCl free Hanks Buffered Salt Solution only.

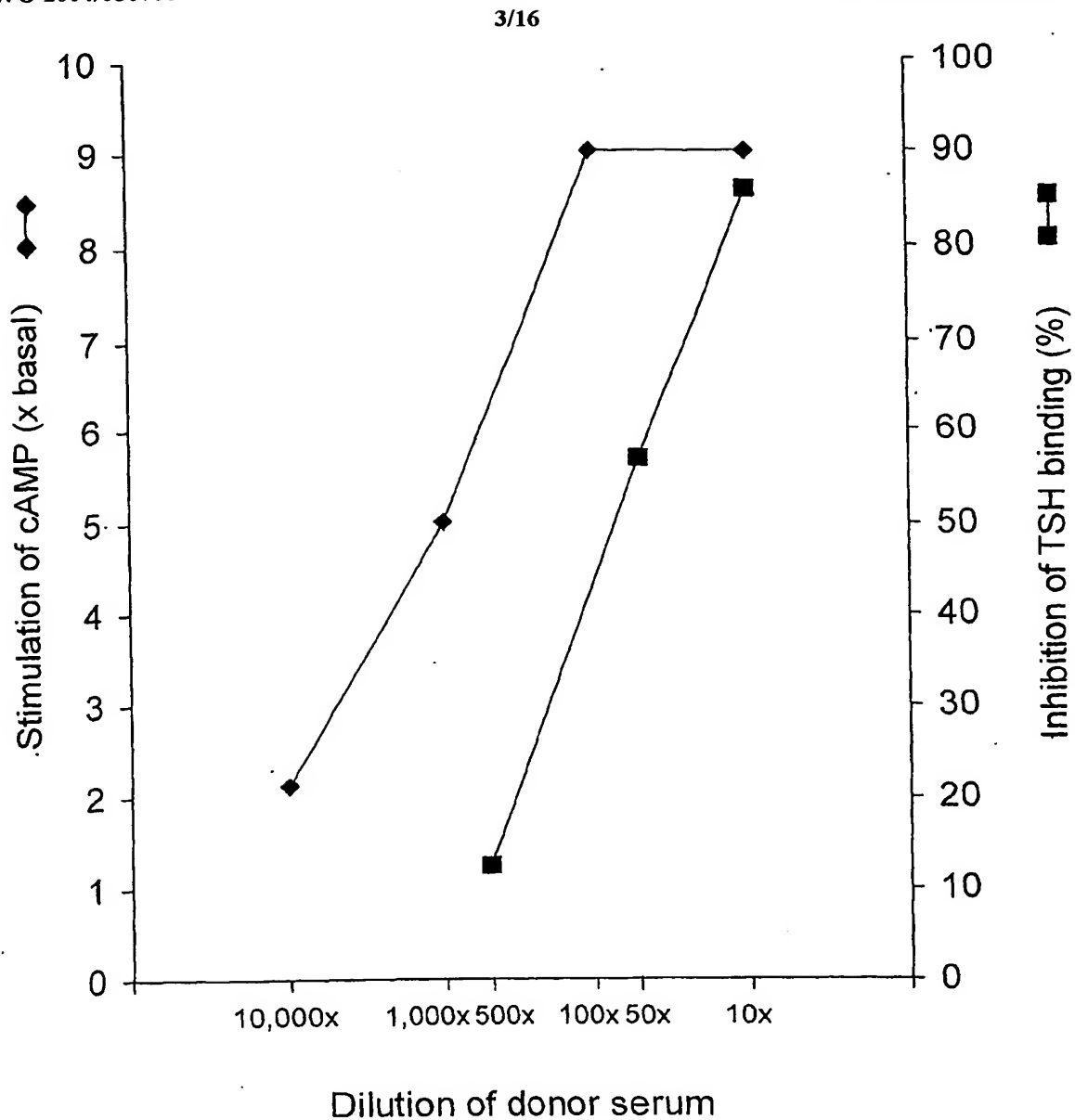
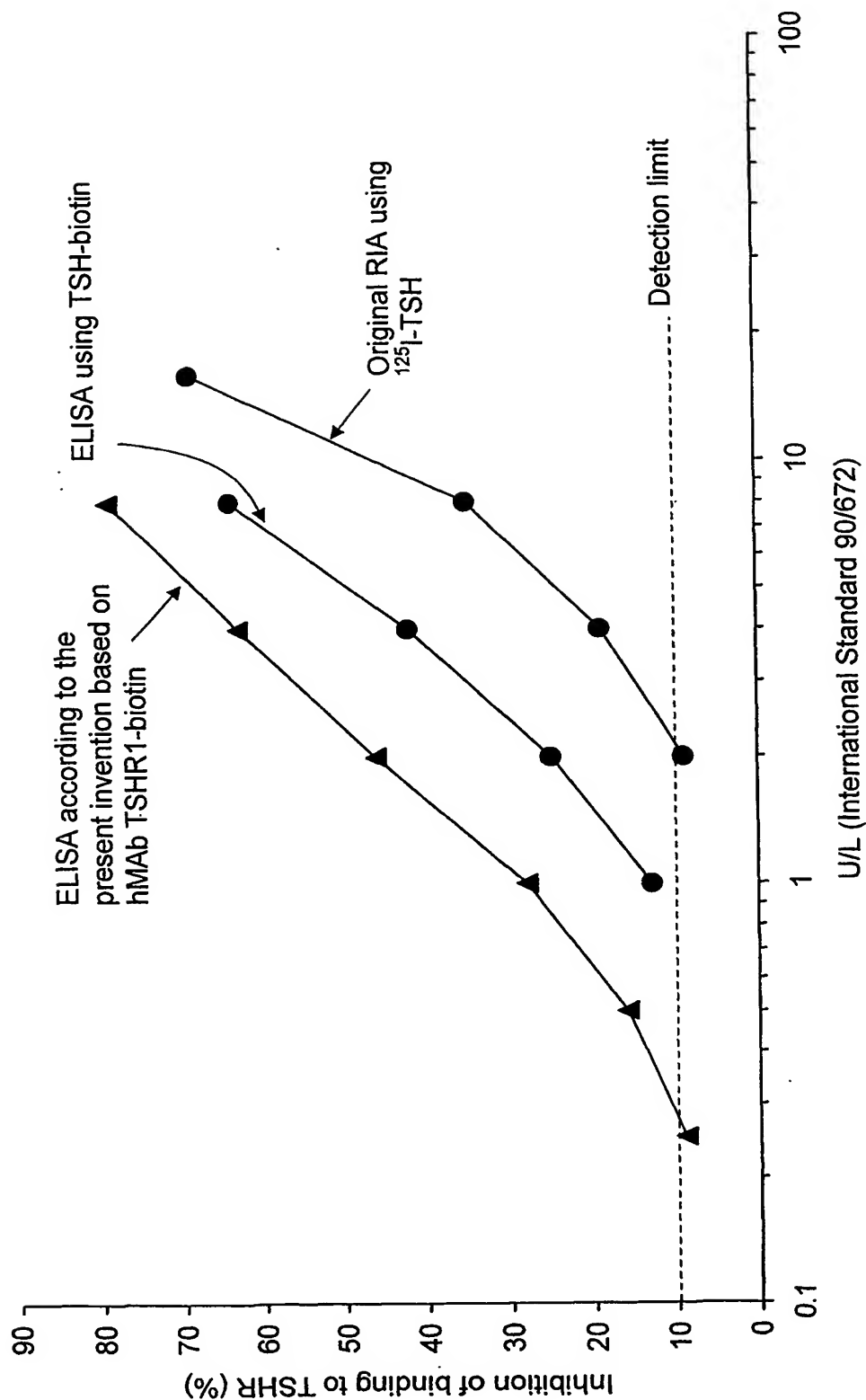


Figure 3 Effect of lymphocyte donor serum on inhibition of TSH binding to the TSHR and on stimulation of cyclic AMP in TSHR transfected CHO cells. In the case of the binding inhibition assay the serum was diluted in a pool of healthy blood donor sera. For the stimulation assay, the serum was diluted in NaCl free Hanks Buffered Salt Solution. Healthy blood donor sera ($n = 3$) gave responses ranging from 1.1 – 1.3 x basal.

Comparison of an ELISA for TSHR autoantibodies according to the present invention with earlier assays. In particular an ELISA based on TSH-biotin described by J Bolton, J Sanders, Y Oda, C Chapman, R Konno, J Furmaniak, B Rees Smith. "Measurement of thyroid-stimulating hormone receptor autoantibodies by ELISA." Clinical Chemistry 1999 volume 45 pp 2285-2287 and the original RIA described by K Southgate, FM Creagh, M Teece, C Kingswood, B Rees Smith. "A receptor assay for the measurement of TSH receptor antibodies in unextracted serum" 1984. Clinical Endocrinology volume 20 pp 539-543.

Figure 3a



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Figure 3b Comparison of an ELISA for TSHR autoantibodies according to the present invention and an ELISA based on TSH-biotin described by J Bolton, J Sanders, Y Oda, C Chapman, R Konno, J Furmaniak, B Rees Smith. "Measurement of thyroid-stimulating hormone receptor autoantibodies by ELISA." Clinical Chemistry 1999 volume 45 pp 2285-2287. Sera from 72 patients with Graves' disease were compared. $y = 1.1154x - 13.032$, $r = 0.99$.

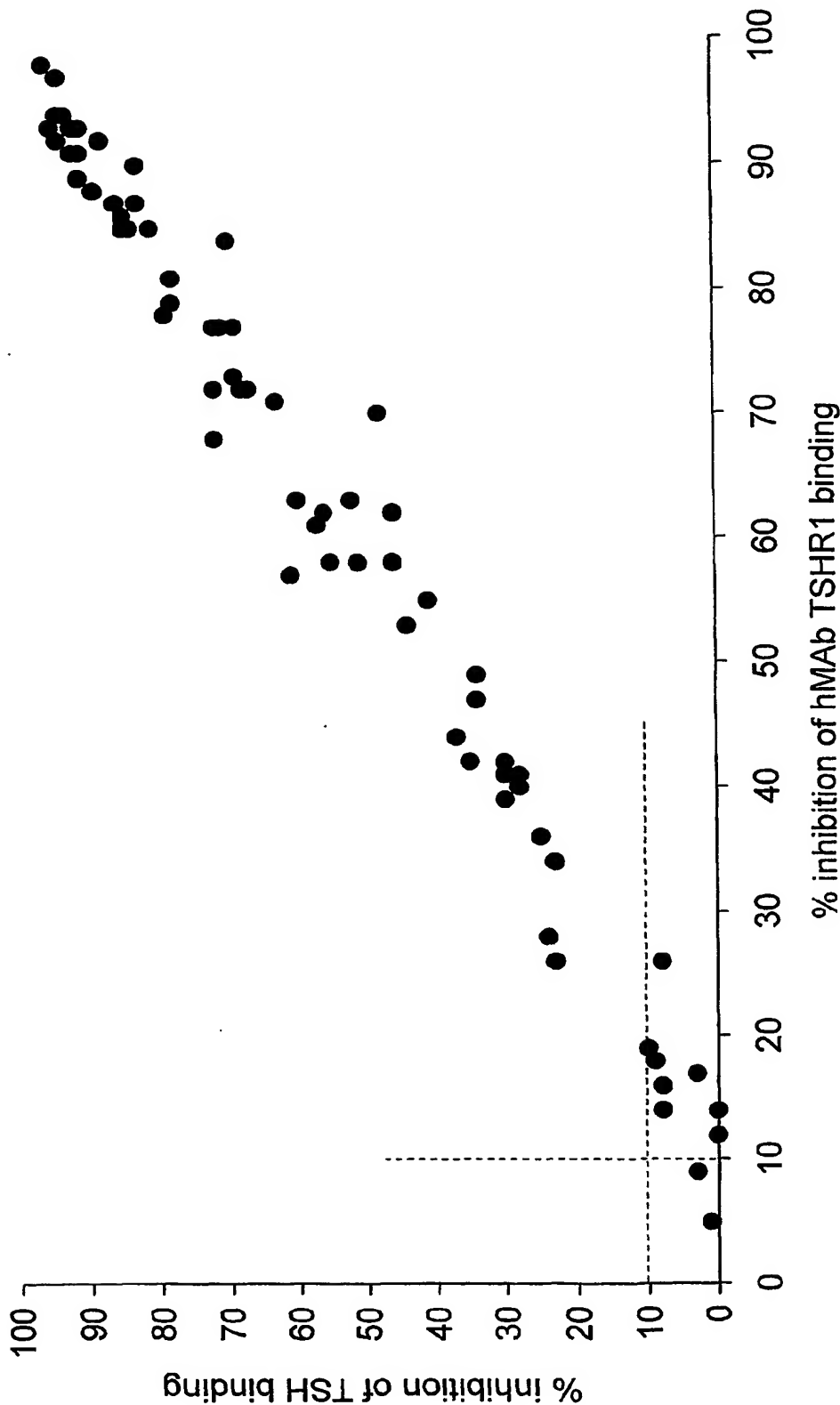


Figure 4 hMAb TSHR1 Heavy Chain V, D and J region nucleotide sequence**Figure 4a**

caaatgcagctggtgcagtctggagcagaggtgaaaaagcccggggagtc
tctgaagatctcctgtaggggttctggatacaggtttaccagctactgga
tcaactgggtgcgccagctgcccggaaggcctagagtggatgggcagg
attgatcctactgactcttataccaactacagtccatccttcaaaggcca
cgtcaccgtctcagctgacaagtccatcaacactgcctacctgcagtgga
gcagcctgaaggcctcggacaccggcatgtattactgtgcgaggctcgaa
ccgggctatagcagcacctggtccgtaaattggggccagggaaccctggt
caccgtctcctcagcctccaccaagggcccatcggtcttcccc

Figure 4b

| | |
|---|-----|
| caaatgcagctggtgcagctctggagcagaggtgaaaaagcccggggagtc | 50 |
| PCR primer | |
| tctgaagatctcctgtaggggttctggatacaggtttaccagctactgga | 100 |
| CDR I | |
| teaatcgggtgcgccagctgcccggaagggcctagagtggatgggagg | 150 |
| CDR II | |
| attgatcctactgactcttataccaatcacagtcctccttcaaaggcca | 200 |
| cgtcaccgtctcagctgacaagtccatcaacactgcctacctgcagtgga | 250 |
| gcagcctgaaggcctcggacaccggcatgtattactgtgcgaggctggaa | 300 |
| CDR III | |
| cgggctatagcagcacctggtcgttaat | 350 |
| constant region | |
| caccgtctcctcagcctccaccaaggggcccatcggtcttcccc | 394 |

Figure 5 hMAb TSHR1 Heavy Chain V, D and J region amino acid sequence

Figure 5a

QVQLVQSGAEVKKPGESLKISCRGSGYRFTSYWINWVRQLPGKGLEWMGR

IDPTDSYTNYSFSFKGHVTVSADKSINTAYLQWSSLKASDTGMYTCARLE

PGYSSTWSVNWGQGLVTVSSASTKGPSVFP

Figure 5b

| | |
|--|-----|
| QVQLVQSGAEVKKPGESLKISCRGSGYRFTSYWINWVRQLPGKGLEWMGR | 50 |
| CDR I | |
| IDPTDSYTNYSFSFKGHVTVSADKSINTAYLQWSSLKASDTGMYTCARLE | 100 |
| CDR II | |
| PGYSSTWSVNWGQGLVTVSSASTKGPSVFP | 131 |
| CDR III | |
| constant region | |

Figure 6 hMAb TSHR1 Light Chain DNA sequence**Figure 6a**

ctgcctgtgctgactcagccaccctcggtgtctggagccccaggcagag
 ggtcaccatctcctgttctggaaacagctccaacatcggaataaatgctg
 taaactggtaccagcagctcccaggaaaggctcccaaactcctcatttat
 tatgatgatcaactgccctcaggggtctctgaccgattctctggctccag
 gtctggcacctccgcctccctggccatccgtgggctccagtctgaggatg
 aggctgattattactgtacatcatgggatgacagcctggatagtcaactg
 ttcggcggaggaggaccaggctgaccgtcctaggt

Figure 6b

| | |
|--|-----|
| ctgcctgtgctgactcagccaccctcggtgtctggagccccaggcagag | 50 |
| PCR primer | |
| ggtcaccatctcctgttctggaaacagctccaacatcggaataaatgctg | 100 |
| CDR I | |
| taaactggtaccagcagctcccaggaaaggctcccaaactcctcatttat | 150 |
| tatgatgatcaactgccctcaggggtctctgaccgattctctggctccag | 200 |
| CDR II | |
| gtctggcacctccgcctccctggccatccgtgggctccagtctgaggatg | 250 |
| aggctgattattactgtacatcatgggatgacagcctggatagtcaactg | 300 |
| CDR III | |
| ttcggcggaggaggaccaggctgaccgtcctaggt | 333 |

Figure 7 hMAb TSHR1 Light Chain protein sequence

Figure 7a

LTVLTQPPSVSGAPRQRVTISCSGNSSNIGNNAVNWYQQLPGKAPKLLIY

YDDQLPSGVSDRFGSRSGTSASLAIRGLQSEDEADYYCTSWDDSLDSQL

FGGGTRLTVLG

Figure 7b

| | | |
|-------------------------|--|-----|
| LTVLTQPPSVSGAPRQRVTISCS | SGNSSNIGNNAVNWYQQLPGKAPKLLIY | 50 |
| CDR I | | |
| YDDQLPS | GVSDRFGSRSGTSASLAIRGLQSEDEADYYCTSWDDSLDSQL | 100 |
| CDR II | CDR III | |
| FGGGTRLTVLG | | 111 |

Figure 8 Effects of 2 patient sera (T1 and T2 with TSH antagonist activity) on stimulation of cyclic AMP production (in CHO cells transfected with the TSHR) by pTSH (0.5 ng/mL) and hMAb TSHR1 IgG (10 ng/mL) and Fab (5 ng/mL)

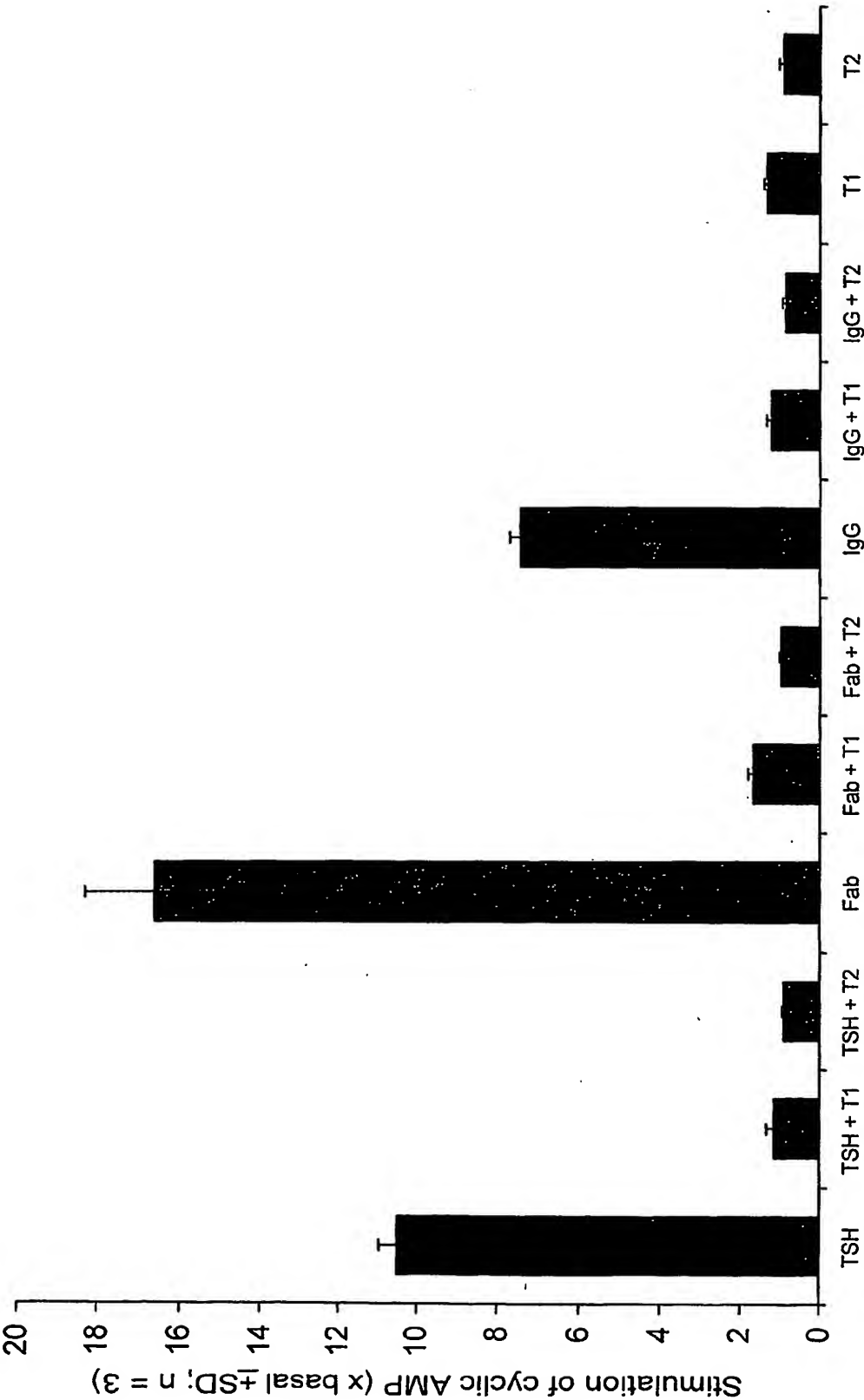


Figure 9 9D33 Heavy Chain nucleotide sequence

Figure 9a

gacgtccagatccagcagcctgggactgagcttgtgaagcctggggcttc
agtgagactgtcctgcaaggcttctggctacaccttcaccacctactgga
tgcaactgggtgaagcagaggcctggacaaggccttgagtggatcggagag
attgatccttctgatagttataactaactataatcaaaagttcaagggcaa
ggccacattgactgtagacaaatcctccagcacagcctacatgcacctca
gcagcctgacatctgaggactctgcggtctattactgttcaagaaactac
ggtagtggctactactttgactactggggccaaggcaccactctcacagt
ctcctcagccaaaacaacacccc

Figure 9b

| | |
|--|-----|
| <u>gacgtccagatccagcagcctgggactgagcttgtgaagcctggggcttc</u> PCR primer | 50 |
| agtgagactgtcctgcaaggcttctggctacaccttcacc <u>acctactgga</u> CDR I | 100 |
| <u>tgcact</u> gggtgaagcagaggcctggacaaggccttgagtggatcgga <u>gag</u> CDR II | 150 |
| <u>attgatccttctgatagttataactataatcaaaagttcaagggc</u> aa | 200 |
| ggccacattgactgtagaca ^{aa} atcctccagcacagcctacatgcacctca | 250 |
| gcagcctgacatctgaggactctgcggtctattactgttcaaga <u>aactac</u> CDR III | 300 |
| <u>ggtagtggctactactttgactact</u> tggggccaaggcaccactotcacagt | 350 |
| ctcctcagc ccaaaacaacacccc constant region | 373 |

Figure 10 9D33 Heavy Chain amino acid sequence

Figure 10a

DVQIQQPGTELVKPGASVRLSCKASGYTFTTYWMHWVKQRPGQGLEWIGE

IDPSDSYTNYNQKFKGKATLTVDKSSSTAYMHLSSLTSEDSAVYYCSRNY

GSGYYFDYWGQGTTLTVSSAKTTP

Figure 10b

| | | | |
|--------------------------------|----------------------------------|-----------------|-----|
| DVQIQQPGTELVKPGASVRLSCKASGYTFT | TYWMH | WVKQRPGQGLEWIGE | 50 |
| PCR primer | CDR I | | |
| IDPSDSYTNYNQKFKG | KATLTVDKSSSTAYMHLSSLTSEDSAVYYCSR | NY | 100 |
| CDR II | | CDR III | |
| GSGYYFDY | WGQGTTLTVSSAKTTP | | 124 |
| | constant region | | |

Figure 11 9D33 Light Chain nucleotide sequence

Figure 11a

ggcgttgagatgacacagtcgccagcaatcatgtctgcatctccagggga
 gaaggtcaccatgacctgcagtgccagctcaagtgttaagttacatgcact
 ggtaccagcagaagtcaggcacctccccaaaagatggatttatgacaca
 tccaaactggcttctggagtccttgctcgcttcagtggcagtgggctctgg
 gacctcttactctctcacaatcagcagcatggagactgaagatgctgcca
 cttattactgccagcagtgaggtagtaaccctggacgttcggtggaggc
 accaaactggaaatcaaacggctgatgctgc

Figure 11b

| | |
|---|-----|
| ggcgttgagatgacacagtcgccagcaatcatgtctgcatctccagggga | 50 |
| PCR primer | |
| gaaggtcaccatgacctgcagtgccagctcaagtgttaagttacatgcact | 100 |
| CDR I | |
| ggtaccagcagaagtcaggcacctccccaaaagatggatttatgacaca | 150 |
| CDR II | |
| tccaaactggcttctggagtccttgctcgcttcagtggcagtgggctctgg | 200 |
| gacctcttactctctcacaatcagcagcatggagactgaagatgctgcca | 250 |
| CDR III | |
| cttattactgcagcagtgaggtagtaaccctggacgttcggtggaggc | 300 |
| accaaactggaaatcaaacggctgatgctgc | 331 |
| constant region | |

Figure 12 9D33 Light Chain amino acid sequence

Figure 12a

GVEMTQSPAIMASAPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDT

SKLASGVPARFSGSGSGTSYSLTISSMETEDAATYYCQQWSSNPWTFGGG

TKLEIKRLML

Figure 12b

| | |
|--|-----|
| GVEMTQSPAIMASAPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDT | 50 |
| PCR primer CDR I | |
| SKLASGVPARFSGSGSGTSYSLTISSMETEDAATYYCQQWSSNPWTFGGG | 100 |
| CDR II CDR III | |
| TKLEIKRLML | 110 |
| constant region | |